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EVIDENCE FOR A NONINTESTINAL NUTRITIONAL MECHANISM IN THE RHYNCHOCOELAN, *LINEUS RUBER*[1]

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The majority of the members of the phylum Rhynchocoela are found free-living in the benthos of the intertidal and subtidal zones. These worm-like organisms are described as acoelomate Bilateria with a complete digestive system (Hyman, 1951). Coe (1943) described the rhynchocoelans (nemerteans) as carnivorous in their feeding behavior, and in some littoral communities these organisms are apparently the most abundant predators (Roe, 1970).

The function and morphology of the digestive system of a number of rhynchocoels has been extensively examined with regard to particulate food digestion by Gontcharoff (1948), Jennings (1960, 1962), Jennings and Gibson (1969) and Gibson (1970) utilizing light microscopy and histochemical techniques.

The ability of numerous free-living marine invertebrates to remove organic solutes, present in relatively low concentrations, from the environment has been extensively investigated by Stephens and his associates (*e.g.*, Stephens, 1964; Reish and Stephens, 1969). Similar absorption phenomena for small molecular weight organic nutrients have been identified in the rhynchocoelan, *Lineus ruber*, by Fisher and Cramer (1967). This paper extends those preliminary reports and indicates that the epidermal free surface is the major site for absorption of these nutrients from their environment.

Materials and Methods

Source and maintenance of animals

Specimens of *Lineus ruber* (5 to 8 cm) were collected at low tide in the vicinity of Manomet Point, Massachusetts, and maintained in the laboratory in running sea water at 20–24° C. Animals were starved while being maintained in artificial sea water lacking organic solutes (Cavanaugh, 1964), and all animals shipped to Rice University for study were held in Instant Ocean (34‰; Aquarium Systems, Inc.). All experimental animals were provided with a coarse sand substrate of sufficient depth to cover the bottom of the holding containers.

Solute accumulation techniques

Rhynchocoelans, being vermiform animals, were manipulated, as described below, after the methods of Fisher and Read (1971); however, artificial sea water

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was substituted for the balanced saline used by those authors for elasmobranch parasites. On removal from preincubation in artificial sea water at 20° C for 30 minutes to 1 hour, individual samples of at least five organisms were carefully blotted on hard surfaced filter paper and transferred to ^{14}C-galactose, ^{14}C-glucose, ^{14}C-leucine or ^{14}C-alanine containing incubation mixture. Unless otherwise stated, the incubation time and temperature were two minutes and 20° C. Following the incubation period the groups of worms were removed from the radioactive mixture, quickly rinsed three times in large volumes of artificial sea water, blotted, weighed on a torsion balance, and placed in 2.0 ml of 70% ethanol. Worm carcasses were extracted in the ethanol with intermittent shaking for at least 18 hours at room temperature (22–26° C). Aliquots of the extraction ethanol were dried on ringed-copper planchettes and counted in a low background gas-flow counter for 10 minutes or 10^4 counts, whichever process ended first. The original incubation solution was diluted 1 : 100 with 70% ethanol and counted in a similar manner to determine the specific activity of the test medium. Ethanol extracted worms were dried to constant weight in aluminum foil tares at 100° C and weighed on an analytical balance. Total water within worms of a single group was estimated from wet weight and dry weight comparisons. These data were used to calculate the internal radiolabeled substrate's concentration represented by μmoles/ml worm water.

Chemical assays

Polysaccharide was determined by the phenol : H_2SO_4 method of Dubois, Gilles, Hamilton, Rebers and Smith (1956) and the modified anthrone method of Dimler, Schaffer and Wise (1952). Glucose was estimated by the glucose oxidase method utilizing the Glucostat Special (Worthington Biochemical Corporation). Protein was isolated by trichloroacetic acid (TCA) precipitation of homogenates of worm carcasses and estimated by the colorimetric method of Lowry, Rosebrough, Farr and Randall (1951).

Chromotagraphic analysis of worm extracts was accomplished using the following solvents on Whatman #1 paper: first) N-butanol : ethanol : acetone : water = 50 : 40 : 30 : 20 (descending) (Gray and Frankel, 1954); secondly, N-butanol : propionic acid : water = 63 : 31 : 44 (descending) (Crowley, 1963); and thirdly, 1-propanol : ethyl acetate : water = 7 : 1 : 2 (ascending) (Baar, 1954). Carbohydrates were visualized on developed chromatograms with the alkaline silver nitrate reagent of Trevelyan, Procter and Harrison (1950). Radioactive areas were localized on chromatograms using a gas-flow scanner. These areas were eluted from chromatograms by a technique similar to that of Dimler *et al.* (1952), and the eluates were reduced to dryness *in vacuo* at 40° C. The redissolved residue was co-chromatographed with authentic standards in the above solvents for positive identification.

Ultrastructural techniques

Specimens of *Lineus ruber* were placed in 4° C, 0.12 M monobasic sodium phosphate-sodium hydroxide buffer at pH 7.4 containing 6% glutaraldehyde plus 3% sucrose. Subsequently the partially fixed worms were cut into cross sections about 1 mm thick and returned to the fixative for three to six hours. Fixation was

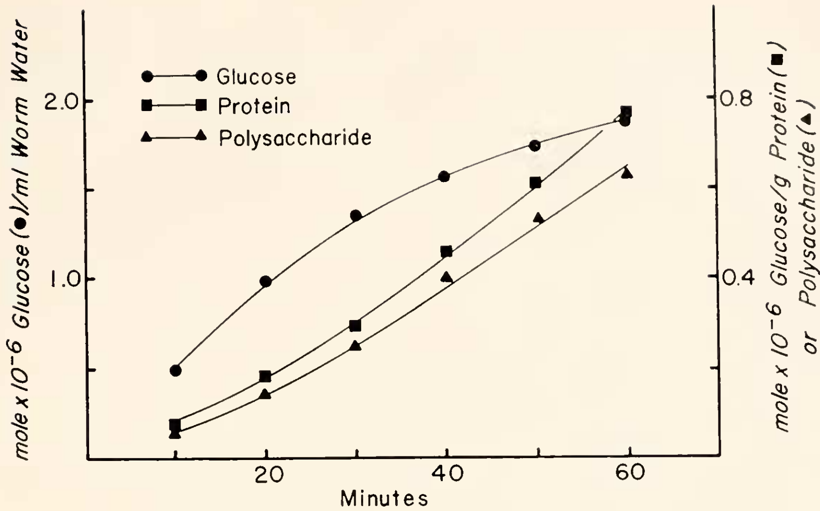

FIGURE 1. The accumulation of 10^{-4} M external glucose into free pool, proteins and polysaccharide of *L. ruber* with time. Glucose in the free pool was determined by glucose oxidase, while calculations of glucose incorporated into protein and polysaccharide were based upon specific activity of radioglucose. Data is based on the mean of four samples per time interval.

terminated by washing in buffer containing 5% sucrose. After washing in buffer for 12 to 18 hours, the tissue was post-fixed for 45 minutes in 1% osmium tetroxide plus 1% sucrose in the same buffer. Post-fixed tissue was rinsed in tap water, dehydrated through a series of ethanol and propylene oxide solutions and embedded in Epon Epoxy 812 (Shell Chemical Co.). Thin sections cut on diamond knives were mounted on bare copper grids, stained with lead and uranium salts and viewed in the Philips 300 electron microscope.

RESULTS

Permeation of glucose

Lineus ruber incubated in sea water containing 10^{-4} M glucose rapidly accumulated that hexose into the free pool within the worm. When extracts were examined, the concentration of glucose inside the worm determined by chemical analysis was ten times that in the external medium after an incubation period of 20 minutes, and within 60 minutes the internal concentration was approximately 17 times the original concentration in the surrounding sea water (Fig. 1). The initial "free" glucose in the worms was less than 2×10^{-4} mole of "worm water." If trace amounts of ^{14}C-glucose (0.1 μCi/μmole) were added to the incubation medium, both protein and polysaccharide fractions of the nemerteans became labeled and the amount of incorporation of the carbon from hexose into these large molecular weight compounds increased up to at least 60 minutes (Fig. 1).

It could be argued that, because these worms possess a digestive system, glucose was ingested from the surrounding sea water and accumulation was occurring across the intestinal epithelium. If these worms were obtaining labeled glucose by

swallowing the sea water medium, one would expect nonuniform distribution of radiocarbon along the linear dimension of the worm body; *i.e.*, during short term incubation periods more label would be absorbed into tissues near the mouth and anterior end of the animal, and, as the length of the incubation period increased, the label would proceed down the gut toward the anus. Individual rhynchocoels were incubated for varying periods of time in sea water containing 10^{-4} M glucose-^{14}C. At the termination of the incubation period the worms were rinsed in sea water, placed in an extended position on a thin glass plate resting on solid CO_2, and frozen immediately. Each worm was cut into 0.5 cm pieces and each section was individually extracted in ethanol for determination of the radioactivity along the length of the nemertean. The data in Table I indicate that there was uniform distribution of label along the length of the nemertean following different incubation periods. In no case was there any difference in the amount of radiocarbon in either end of the worm which suggests that over the time period examined, movement of sea water into the gut *via* the mouth and/or the anus is not a significant factor in determining the distribution of accumulated glucose. These data also suggest that glucose accumulation is occurring over the entire surface of the worm and that there is no difference in the rate of glucose influx along the body of the rhynchocoelan. Confirmation that the worms were not ingesting the incubation medium was obtained by ligaturing ten specimens of *Lineus ruber* posterior to the mouth and anterior to the anus with 4/0 silk suture prior to the incubation in the radioactive substrate. No difference in distribution of accumulated radioglucose could be observed between ligatured and the unligatured control animals (Table II). Amounts of radioglucose/body section is similar in control and ligatured animals (Table II).

The initial rate of glucose accumulation was examined during two-minute incubations using glucose concentrations ranging over several orders of magnitude. Glucose influx exhibits initial rate saturation kinetics as a function of concentration

TABLE I

Distribution of ethanol-soluble counts/minute along the body of Lineus ruber *after incubation for different periods. Initial external glucose-^{14}C concentration is 10^{-4} M; incubation temperature, 20° C.*

Number of section	counts/minute				
	1 min	2 min	4 min	8 min	16 min
1 anterior	90	160	201	340	873
2	73	171	260	339	734
3	89	163	220	370	759
4	69	181	229	310	819
5	91	170	248	319	840
6	83	190	271	357	839
7	94	167	209	348	790
8	71	159	219	301	763
9	75	179	210	359	793
10	84	187	254		779
11	70		220		810
12 posterior	61				791

TABLE II

Distribution of ethanol-soluble counts/minute along the body of ligatured and unligatured Lineus ruber. *Initial external glucose-^{14}C concentration is 10^{-4} M; incubation time, 5 minutes at 20° C.*

Number of section	$\bar{x}$ counts/minute (± standard error of the mean)	
	Ligatured	Without ligature
1 anterior	263 ± 47	258 ± 47
2	243 ± 39	261 ± 51
3	199 ± 61	241 ± 32
4	231 ± 27	229 ± 26
5	246 ± 43	263 ± 59
6	271 ± 59	237 ± 23
7	251 ± 47	231 ± 31
8	220 ± 17	243 ± 35
9	237 ± 30	240 ± 40
10	246 ± 38	221 ± 26
11	251 ± 53	279 ± 60
12 posterior	239 ± 37	264 ± 61
	n = 10	n = 15

(Fig. 2). Galactose uptake followed a similar entry pattern (Fig. 2). The accumulation of glucose is inhibited by galactose (66% inhibition) and three glycosides: phlorizin (93% inhibition), quercetin (71% inhibition) and ouabain (29% inhibition). Phlorizin, the β-glucoside of phloretin and β-D-glucose, is a potent inhibitor of glucose permeation in a number of systems (see Crane, 1960). The permeation of glucose was not inhibited by other hexoses, or di- and trisaccharides including levulose, mannose, N-acetylglucosamine, cellobiose, maltose, trehalose, sucrose and melibiose. Amino acids, fatty acids and organic acids also failed to

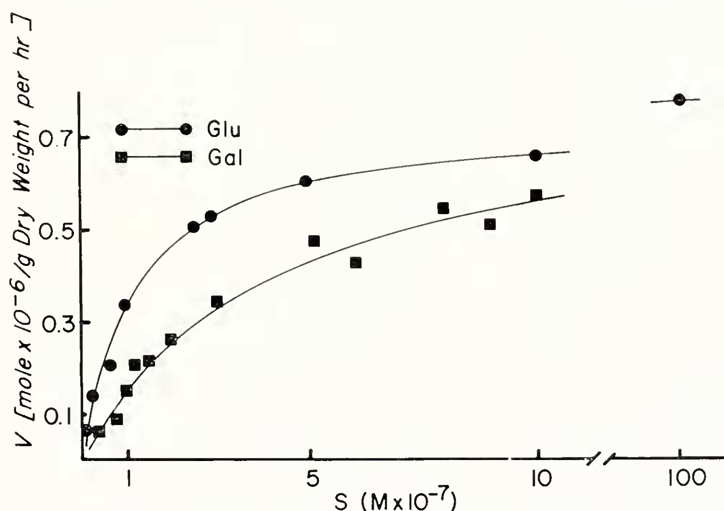

FIGURE 2. The effect of external substrate concentration on the permeation of glucose and galactose into the free hexose pool. Data is based on the mean of three samples per concentration of hexose.

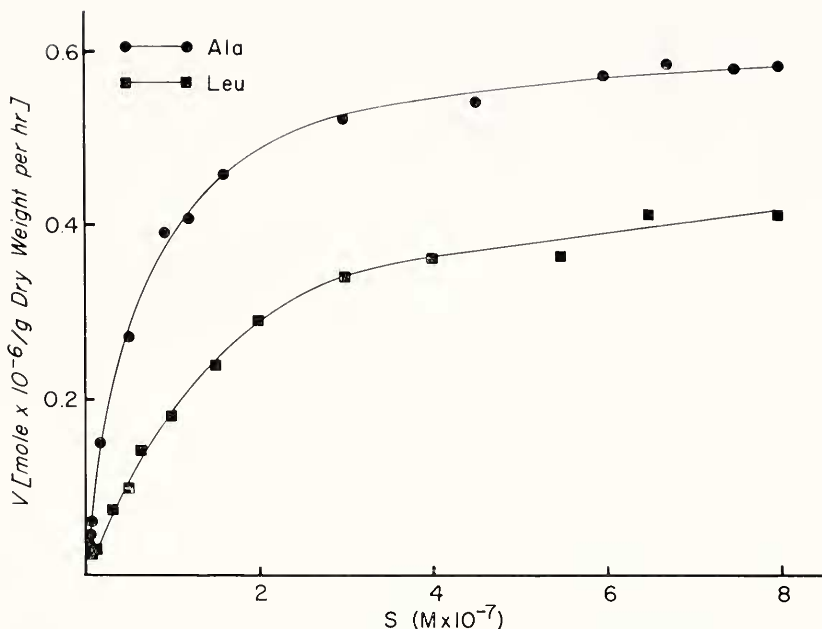

FIGURE 3. The effect of external substrate concentration on the accumulation of alanine and leucine into free pool of *L. ruber*. Data is based on the mean of three determinations per concentration of amino acid.

inhibit glucose permeation. In these experiments the initial external glucose concentration was 10^{-4} M; and the inhibitors, 10^{-3} M. All incubations were carried out for two minutes at 20° C.

Chromatographic examination of ethanolic extracts of worms after two-minute incubation in radioglucose revealed that there was very little (<0.5%) metabolism of the glucose during the relatively short incubation period. If nemerteans are post-incubated in sea water without glucose following a two-minute incubation in radioglucose, there is no chromatographically identifiable glucose "leakage" into the efflux medium. There are, however, traces of succinate which appear in the surrounding medium during this second incubation period.

Permeation of alanine and leucine

The uptake of alanine and leucine by *Lineus ruber* also followed saturation kinetics (Fig. 3). The fate of the accumulated alanine was further examined using long-term incubations to follow the possible appearance of radiocarbon into protein. The results of this experiment, expressed as μmoles/g protein is seen in Figure 4. The radiocarbon skeleton from alanine was incorporated into protein during the 60-minute experimental period. Longer incubation times were not examined.

The accumulation of alanine and leucine were not influenced by 50:1 ratios of carbohydrates, organic acids or fatty acids; however, the uptake of those substrates was inhibited by some amino acids examined (Table III). Diabasic and dicarboxylic amino acids, the imino acid proline, and the sulfonic acid derivative, taurine,

TABLE III

Inhibition of alanine and leucine by other l-amino acids. External substrate concentration is 5 × 10⁻⁵ M; inhibitor concentration, 2 × 10⁻³ M; incubation time, 2 minutes at 20° C.

Inhibitor	Inhibition of alanine permeation (%)	Inhibition of leucine permeation (%)
Alanine	97	49
β-Alanine	11	0
Arginine	0	0
Aspartic acid	0	0
Glutamic acid	0	0
Glycine	64	39
Isoleucine	19	53
Leucine	53	94
Lysine	0	0
Methionine	47	61
Proline	0	0
Taurine	0	0
Valine	21	49

did not influence the uptake of either alanine or leucine. The mutual inhibition of uptake by leucine and alanine suggested that those amino acids may compete for entry through the same membrane site.

Chromatographic examination of extracts from two-minute incubations indicated that there were no detectable metabolites of either alanine or leucine in the free pool of solutes within the worm bodies. Similar examination of the incubation medium revealed that there were no metabolites of these amino acids excreted into the external medium during the two-minute exposure to the isotopes.

Morphology of the epidermal and epithelial surfaces

Two interfaces for the accumulation of organic solutes exist on most free-living, aquatic metazoans: the epidermal covering and the epithelia lining the digestive

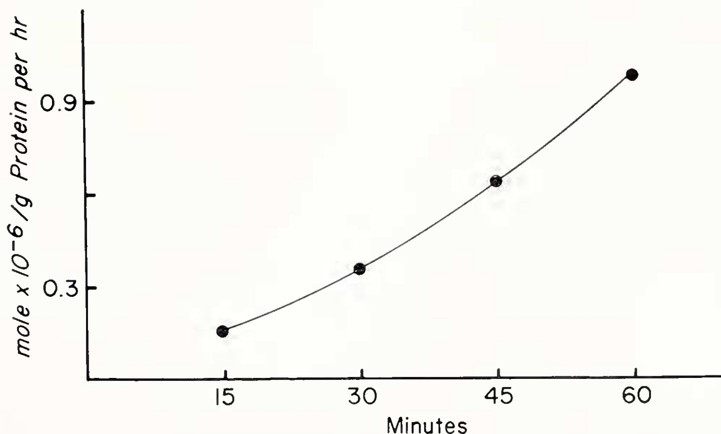

FIGURE 4. The effect of time on the incorporation of 10^{-5} M external alanine into protein of *L. ruber*. Data based on the mean of four samples per time interval.

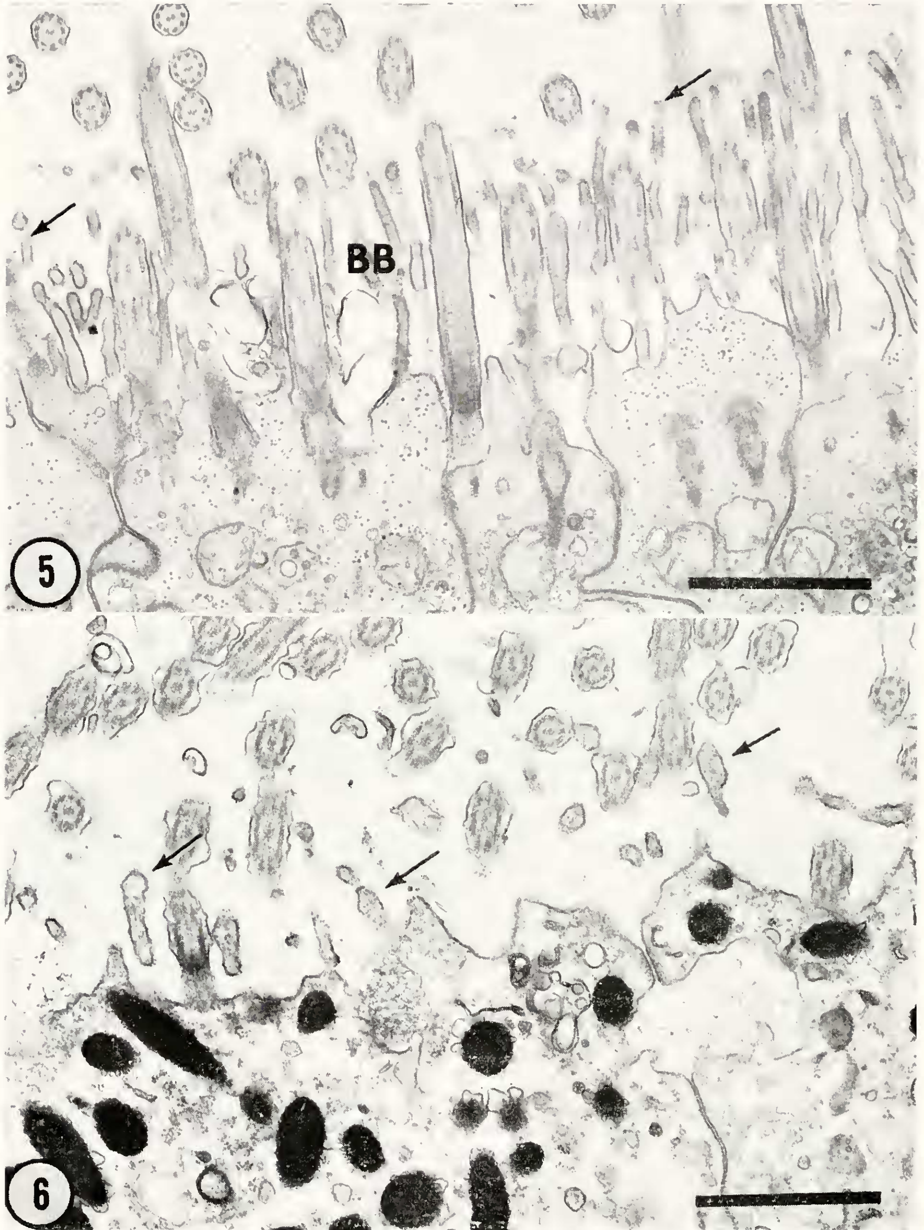

FIGURE 5. Ultrastructure of the external surface of *Lineus ruber* epidermis. Apical cytoplasm and plasmalemma of the free surface possess cilia which extend beyond the outer limits of the brush border (BB), defined by the tips of the microvilli (arrows). Note the close spacing of adjacent microvilli. Bar equals 1 μm.

FIGURE 6. Ultrastructure of the luminal surfaces of *Lineus ruber* gut. Apical cytoplasm and plasmalemma of the intestinal free surface possess a sparse population of microvilli (arrows) projecting from the intestinal surface. Note that these microvilli are not arranged in a parallel array as observed at the epidermal surface. Bar equals 1 μm.

system. Both epithelial surfaces of *Lineus* were examined for structural specialization which could account for the uptake of the dissolved compounds. The free surfaces of the gut epithelium and of the epidermis possess microvilli and cilia amplifing their free surface (Figs. 5, 6). However, the microvilli are far more numerous and regularly distributed on the apical surfaces of epidermal cells than they are on the corresponding surfaces of the intestinal epithelial cells (Fig. 5). The close register of microvilli at the epidermal surface resembles that described for the brush borders present at the surfaces of organs and tissues modified for absorptive function. In contrast to the epidermis, the distribution of microvilli at the surface of the intestinal epithelium in *Lineus* is relatively sparse and less consistently organized (Fig. 6) resembling the surfaces where the function of surface amplification is not well understood (*i.e.,* vertebrate trachea, Steinman, 1968; or the trematode miracidium, Wilson, 1969).

Discussion

There are diverse opinions on the role of dissolved reduced carbon compounds as a source of energy for marine metazoans. Stephens (1967) pointed out that the concept of the utilization of dissolved organic material in the nutrition of aquatic animals is not new and that Pütter (1908a, b) first advanced the idea of their importance. Later Krogh (1931) dismissed this notion and concluded that there was no substantial evidence to support Pütter's hypothesis.

Investigators, too numerous to completely list here, have since shown that various soft-bodied marine invertebrates can remove dilute organic solutes from sea water (*i.e.,* Stephens, 1968), some at concentrations as low as 3×10^{-9} M (Goreau, Goreau, and Yonge, 1971). Johannes, Coward and Webb (1969) criticized the methodology involved in most uptake studies using amino acids because the net efflux of free amino acids has seldom been measured. The simultaneous movements of such compounds into and out of biological systems is well documented. Wilbrandt and Rosenberg (1961) and Johannes *et al.* (1969) stress that there is a net loss of free amino acids during most uptake experiments involving radioactive substrates. However, the significant increase of internal free glucose in *Lineus ruber* incubated in 10^{-4} M glucose suggests that the amount of glucose lost by efflux is relatively small in comparison to the accumulated glucose available to the worm's metabolism. Therefore, some organic solutes available in the organism's environment could serve as a significant source of nutrition for this nemertean.

During the two-minute incubation period used to determine initial rates of glucose accumulation, there are no metabolites of glucose excreted into the incubation medium, and less than 0.5% of the radiocarbon inside the worm is identifiable as nonglucose moieties. During a longer incubation, however, there is metabolism of glucose to succinate. This latter metabolite subsequently appears in the external sea water, and the concentration of this acidic end product increases with extended incubation periods. Absorbed glucose is readily incorporated into the nemertean's polysaccharide and almost equivalent amounts of radiocarbon are incorporated into the proteins of the worm body. Preliminary analysis of the TCA precipitable fraction indicates that the radiocarbon is present primiarily as alanine with a small amount of aspartic acid suggesting that one pathway of glucose metabolism in *L. ruber* may resemble those reported for a number of parasitic platyhelminths

(Von Brand, 1966). These data also strongly indicate that this hexose absorbed from the surrounding sea water is, in fact, serving as an energy source for this organism. It should be emphasized that the concentration of glucose used in these experiments is consistent with the values reported for dissolved carbohydrate in oceanic waters (Wangersky, 1952; Wangersky and Guillard, 1960; Walsh, 1965a, b, 1966; Walsh and Douglass, 1966).

Absorbed alanine enters the metabolic systems of the worm, since it is readily incorporated into the TCA precipitable fraction during incubation periods of moderate length. Although preliminary in nature, these data indicate that amino acids from the surrounding sea water can and do serve as a source of amino-nitrogen for protein synthesis. The concentration of substrates used in these experiments are also within the range of those reported for oceanic and estuarine waters (Adams and Richards, 1968; Belser, 1959 and 1963; Chau and Riley, 1966; Siegel and Degens, 1966; Webb and Johannes, 1967).

Stephens and Schinske (1961) described the removal of amino acids from sea water by numerous invertebrates belonging to eleven phyla. In their experiments only arthropods failed to remove such solutes from the surrounding water. The presence of a hard, acellular, relatively impermeable cuticle on the exterior of arthropods and an epidermis on soft-bodies marine invertebrates suggests that the epidermis may be the site for absorption of some soluble substances from sea water. The observed morphology of the external surface of *Lineus ruber* is typical of many of those soft-bodied organisms. MacRae (1967) has also found brush border microvilli amplifying the epidermal surface area in contact with sea water on Turbellaria; Lloyd (1969) and Lane (1963) on molluscs; Little and Gupta (1968) and Norrevang (1965) on pogonophorans; Potswald (1971) on annelids; and Menton and Eisen (1970) on echinoderms. Except for the cilia present among the brush border's microvilli in some invertebrate epidermises, these brush borders resemble those on tissue surfaces known to possess high rates of transport of amino acids and monosaccharides, such as the vertebrate intestine, the proximal tubule of the kidney and the tegument of tapeworms (reviewed by Lumsden, 1975). The resemblance of these surfaces is also consistent with the hypothesis that the epidermis covering *Lineus ruber* may be the site of absorption of nutrient molecules present in sea water. The epithelium of the digestive system in *Lineus ruber* also possesses microvilli. These could serve as a second site of solute absorption from sea water, even though they are fewer in number on the intestinal surface than those present on the epidermis. Jennings (1969) suggests that the intestinal epithelium serves to phagocytize partially digested material from the lumen of the gut rather than as a primary surface for nutrient solute transport. In line with that suggestion, our experiments, involving the use of ligatured and unligatured nemerteans, indicates that the epidermis investing this worm is the most important, if not the sole route, in solute feeding.

In a preliminary report, Fisher and Cramer, (1967) suggested that the membrane transport of solutes represented a new feeding mechanism in the phylum Rhynchocoela. We have shown that glucose enters *L. ruber* by a mediated process. Our data and that of Fisher (unpublished) suggest that the accumulation of glucose is competitively inhibited by galactose. Three glucosides also inhibit glucose

permeation; however, amino acids, fatty acids and organic acids are without effect. This accumulation process for glucose can be described as an active transport system (Fisher, unpublished). The facts that the concentration of chemically determined glucose inside the worm is greater than that in the external sea water, that a stereoisomer of glucose inhibits uptake and that glucose is accumulated against a concentration gradient also support the notion that this is a mediated process.

Alanine and leucine also enter *L. ruber* by a mediated process which demonstrates saturation kinetics. Entry of these compounds is inhibited by other neutral amino acids; however, acidic and basic amino acids, as well as proline and taurine, do not inhibit the uptake of alanine and leucine. Our data and that of Fisher (unpublished) indicate that the uptake of alanine is competitively inhibited by leucine.

The undiminished accumulation of glucose in ligatured and nonligatured animals, the incorporation of glucose into polysaccharide, the synthesis of amino acids from hexose with subsequent incorporation into protein, the incorporation of an absorbed amino acid into protein fraction, and the consistency of the epidermal morphology with other tissues which are known to transport solutes, strongly support the notion that this surface of *Lineus ruber* serves as a functional feeding mechanism, capable of metabolite accumulation from sea water in its littoral habitat.

Summary

1. *Lineus ruber* rapidly accumulates glucose from sea water into free pools within the worm concentrating the hexose to 17 times the original external concentration (10^{-4} M) in one hour.

2. Accumulated glucose, alanine and leucine are incorporated into protein, and additional glucose is incorporated into polysaccharide. No free glucose, alanine or leucine is effluxed during two minutes; however, succinate, derived from glucose, is detectable in the external medium when the incubation time is extended.

3. The demonstration of saturation kinetics for both glucose and galactose, the partial inhibition of glucose entry by galactose and inhibition of glucose accumulation by phlorizin, quercetin and ouabain is consistent with specific sites of glucose transport.

4. Similar kinetics for both alanine and leucine accumulation, their mutual competition for entry and the inability of carbohydrates, organic acid, and fatty acids to influence the uptake of alanine and leucine is consistent with specific transport sites for neutral amino acids.

5. Comparison of glucose accumulation by whole ligatured and unligatured worms, as well as along the length of unligatured worms, indicates that a majority of the sites of entry available to glucose in the worm's environment is through its epidermis.

6. Ultrastructural examination of free epidermal and gut luminal surfaces reveal that each is bounded by a plasmalemma with a surface area expanded by microvilli. The surface area of epidermis is greatly increased by numerous microvilli arranged in the form of a brush border and is greater than the analogous surface region of the gut. Presence of a brush border is characteristic of tissues with high rates of transport function.

LITERATURE CITED

Adams, D. D., and F. A. Richards, 1968. Dissolved organic matter in an anoxic fjord with special reference to the presence of mercaptans. *Deep Sea Res.*, **15**: 471–481.

Baar, S., 1954. Estimation of glucose by paper partition chromatography. *Biochem. J.*, **58**: 175–176.

Belser, W. L., 1959. Bioassay of organic micronutrients in the sea. *Proc. Nat. Acad. Sci. U.S.A.*, **45**: 1533–1542.

Belser, W. L., 1963. Bioassay of trace substances. Pages 220–231 in E. D. Goldberg, Ed., *The Sea, ideas and observations on progress in the study of the seas, Vol. 2 Marine chemistry*. John Wiley and Sons, New York.

Cavanaugh, G. M., 1964. *Formulae and methods of the Marine Biological Laboratory, Vol. V.* Marine Biological Laboratory, Woods Hole, 87 pp.

Chau, Y. K., and J. P. Riley, 1966. The determination of amino acids in sea water. *Deep Sea Res.*, **13**: 1115–1124.

Coe, W. R., 1943. Biology of the nemertines of the Atlantic Coast of North America. *Trans. Conn. Acad. Arts Sci.*, **34**: 59–61.

Crane, R. K., 1960. Intestinal absorption of sugars. *Physiol. Rev.*, **40**: 789–825.

Crowley, G. J., 1963. Studies in arthopod serology. I. Changes in hemolymph composition as related to ecdysal cycle. *Wasmann J. Biol.*, **21**: 177–191.

Dimler, R. J., W. C. Schaffer, and C. S. Wise, 1952. Quantitative paper chromatography of D-glucose and its oligosacchardies. *Anal. Chem.*, **24**: 1411–1413.

Dubois, M., R. R. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, 1956. Colorimetric method of the determination of sugars and related substances. *Anal. Chem.*, **28**: 350–356.

Fisher, F. M., Jr., and N. Cramer, 1967. New observation on the feeding mechanisms in *Lineus ruber* (Rhynchocoela). *Biol. Bull.*, **133**: 464.

Fisher, F. M., Jr., and C. P. Read, 1971. Transport of sugars in the tapeworm *Calliobothrium verticillatum*. *Biol. Bull.*, **140**: 46–62.

Gibson, R., 1970. The nutrition of *Paranemertes peregrina* (Rhynchocoela: Hoplonermertea). II. Observations on the structure of the gut and proboscis, site and sequence of digestion, and food reserves. *Biol. Bull.*, **139**: 92–106.

Gontcharoff, M., 1948. Note sur l'alimentation de quelques nemertes. *Sci. Progr. Natur. Paris*, **10**: 75–78.

Goreau, T. F., N. I. Goreau, C. M. Yonge, 1971. Reef corals: autotrophs or heterotrophs. *Biol. Bull.*, **141**: 247–260.

Gray, H. E., and G. Frankel, 1954. Fructomaltose, a recently discovered trisaccharide isolated from honeydew. *Science*, **118**: 304–305.

Hyman, L. H., 1951. *The invertebrates: Platyhelminthes and Rynchocoels. The acoelomate Bilateria, Vol. II.* McGraw Hill, New York, 460 pp.

Jennings, J. B., 1960. Observations on the nutrition of the rhynochocoelan *Lineus ruber* (O. F. Müller). *Biol. Bull.*, **122**: 63–72.

Jennings, J. B., 1962. A histochemical study of digestion and digestive enzymes in the rhynchocoelan, *Lineus ruber* (O. F. Müller). *Biol. Bull.*, **122**: 63–72.

Jennings, J. B., 1969. Ultrastructural observations on the phagocytic uptake of food materials by the ciliated cells of the rhynchocoelan intestine. *Biol. Bull.*, **137**: 476–485.

Jennings, J. B., and R. Gibson, 1969. Observations on the nutrition of seven species of rhynchocoelan worms. *Biol. Bull.*, **136**: 405–433.

Johannes, R. E., S. J. Coward and R. L. Webb, 1969. Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.*, **29**: 275–281.

Krogh, A., 1931. Dissolved substances as food of aquatic animals. *Biol. Rev.*, **6**: 412–442.

Lane, N. J., 1963. Microvilli on the external surfaces of gastropod tentacles and body walls. *Q. J. Microsc. Sci.*, **104**: 495–504.

Little, C., and B. L. Gupta, 1968. Pogonophora: uptake of dissolved nutrients. *Nature*, **218**: 873–874.

Lloyd, D. C., 1969. Some observations on the skin of *Oxychilus* spp.. (Fitzinger), with particular reference to *O. helveticus* (Blum) (Mollusca, Pulmonata, Aonitidae). *Protoplasma*, **68**: 327–339.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275.

Lumsden, R. D., 1975. Surface ultrastructure and cytochemistry of parasitic helminths. *Exp. Parasitol.*, **37**: 267–339.

MacRae, E. K., 1967. The fine structure of sensory receptor processes in the auricular epithelium of the planaria, *Dugesia tigrina*. *Z. Zellforsch. Mikrosk. Anat.*, **82**: 479–494.

Menton, D. N., and A. Z. Eisen, 1970. The structure of the integument of the sea cucumber, *Thyone briareus*. *J. Morphol.*, **131**: 17–36.

Norrevang, A., 1965. Structure and function of the tentacles and pinnules of *Siboglinum ekmai* Jägersten (Pogonophora) with special references to the feeding problem. *Sarsia*, **21**: 37–47.

Potswald, H. E., 1971. A fine structural analysis of the epidermis and cuticle of the oligochaete, *Acolosoma bengalense* Stephensen. *J. Morphol.*, **135**: 185–212.

Pütter, A., 1908a. Die Ernahrung der Wassertier. *Z. Allg. Physiol.*, **1**: 284–320.

Pütter, A., 1908b. Die Stoffhaus Halt des Meeres. *Z. Allg. Physiol.*, **1**: 321–368.

Reish, D. J., and G. C. Stephens, 1969. Uptake of organic material by aquatic invertebrates. V. The influences of age on the uptake of glycine-C14 by the polychaete *Neanthes arenaceodentata*. *Mar. Biol.*, **3**: 352–355.

Roe, P., 1970. The nutrition of *Paranemertes peregrina* (Rhynchocoela: Hoplonermertea). I. Studies on food and feeding behavior. *Biol. Bull.*, **139**: 80–91.

Siegel, A., and E. T. Degens, 1966. Concentration of dissolved amino acids from saline waters by ligand-exchange chromatography. *Science*, **151**: 1097–1101.

Steinman, R. M., 1968. An electron microscopic study of ciliogenesis in developing epidermis and trachea in the embryo of *Xenopus levis*. *Am. J. Anat.*, **122**: 19–56.

Stephens, G. C., 1964. Uptake of organic material by aquatic invertebrates. III. Uptake of glycine by brackish water annelids. *Biol. Bull.*, **126**: 150–162.

Stephens, G. C., 1967. Dissolved organic material and a nutritional source for marine and estuarine invertebrates. *Am. Assoc. Adv. Sci. Publ.*, **83**: 367–373.

Stephens, G. C., 1968. Dissolved organic matter as a potential source of nutrition for marine animals. *Am. Zool.*, **8**: 95–106.

Stephens, G. C., and R. A. Schinske, 1961. Uptake of amino acids by marine invertebrates. *Limnol. Oceanogr.*, **6**: 175–181.

Trevelyan, W. E., P. Procter, and J. S. Harrison, 1950. Determination of sugars on paper chromatograms. *Nature*, **166**: 444–445.

Von Brand, T., 1966. *Biochemistry of parasites*. Academic Press, New York, 429 pp.

Walsh, G. E., 1965a. Studies on dissolved carbohydrates in Cape Cod waters. I. General survey. *Limnol. Oceanogr.*, **10**: 570–576.

Walsh, G. E., 1965b. Studies on dissolved carbohydrate in Cape Cod waters. II. Diurnal fluctuation in Oyster Pond. *Limnol. Oceanogr.*, **10**: 577–582.

Walsh, G. E., 1966. Studies on dissolved carbohydrate in Cape Cod waters. III. Seasonal variation in Oyster Pond and Wequaquet Lake, Massachusetts. *Limnol. Oceanogr.*, **11**: 249–256.

Walsh, G. E., and J. J. Douglass, 1966. Vertical distribution of dissolved carbohydrate in the Sargasso Sea of Bermuda. *Limnol. Oceanogr.*, **11**: 406–408.

Wangersky, P. J., 1952. Isolation of ascorbic acid rhamnosides from sea water. *Science*, **115**: 685.

Wangersky, P. J., and R. R. L. Guillard, 1960. Low molecular weight organic base from the dinoflagellate *Amphidinium carteri*. *Nature*, **185**: 689–690.

Webb, K. L., and R. E. Johannes, 1967. Studies of the release of dissolved free amino acids by marine zooplankton. *Limnol. Oceanogr.*, **12**: 376–382.

Wilbrandt, W., and T. Rosenberg, 1961. The concept of carrier transport and its corollaries in pharmacology. *Pharmacol. Rev.*, **13**: 109–132.

Wilson, R. A., 1969. Fine structure of the tegument of the miracidium of *Fasciola hepatica* L. *J. Parasitol.*, **55**: 124–133.